

**Data Evaluation Report on the Acute Dietary Toxicity of Copper Sulfate Pentahydrate to Avian Species *Serinus canaria***

PMRA Submission Number {.....}


EPA MRID Number 49535701

<b>Data Requirement:</b>	PMRA Data Code	{.....}
	EPA DP Barcode	D425081
	OECD Data Point	{.....}
	EPA MRID	49535701
	EPA Guideline	OCSPP 850.2200

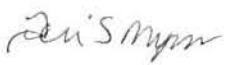
**Test material:** Copper Sulfate Pentahydrate  
**Common name:** Copper Sulfate Pentahydrate  
**Chemical name:** IUPAC: Copper sulfate  
CAS name: Copper Sulfate Pentahydrate  
CAS No.: 7758-99-8  
Synonyms: None reported

**Purity:** 25.55% (Cu)

**Primary Reviewer:** John Marton, Ph.D.  
Environmental Scientist, CDM Smith

**Signature:**   
**Date:** 04/08/15

**Secondary Reviewer:** Teri S. Myers, Ph.D.  
Environmental Scientist, CDM Smith

**Signature:**   
**Date:** 04/20/15

**Primary Reviewer:** Meghan Radtke, Ph.D.  
Biologist, EPA/OPP/EFED/ERB-1

**Date:** 5/18/15 

**Secondary Reviewer(s):** {.....}  
{EPA/OECD/PMRA}

**Date:** {.....}

**Reference/Submission No.:** {.....}

**Company Code** {.....} [For PMRA]  
**Active Code** {.....} [For PMRA]  
**Use Site Category:** {.....} [For PMRA]  
**EPA PC Code** 024401

**Date Evaluation Completed:** 13-05-2015

**CITATION:** Hubbard PM, Schutt WR, Beavers JB. 2014. Copper Sulfate Pentahydrate: A Dietary LC<sub>50</sub> Study with the Canary. Unpublished study performed by Wildlife International, Easton, Maryland. Laboratory report number 241-102. Study sponsored by Copper Sulfate Task Force, Valdosta, Georgia. Study completed December 18, 2014.

**DISCLAIMER:** This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute dietary toxicity of a pesticide to avian species. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

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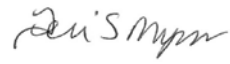
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<b>Use Site Category:</b>	{.....}	[For PMRA]
<b>EPA PC Code</b>	024401	

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## **EXECUTIVE SUMMARY:**

The acute dietary toxicity of copper sulfate pentahydrate (25.55% copper) to 5-33-month-old canaries (*Serinus canaris*) was assessed over 8 days. Copper sulfate pentahydrate was administered to the birds in the diet at nominal doses of 0 (negative control), 128, 227, 404, 718, and 1278 mg Cu/kg diet (corrected for purity by reviewer). These corresponded to nominal doses of 500, 890, 1580, 2810, and 5000 mg/kg diet based on copper sulfate pentahydrate. The reviewer-calculated mean-measured concentrations were 96, 234, 418, 635, and 1076 mg Cu/kg diet, and 377, 916, 1635, 2485, and 4213 mg test material/kg diet. The 8-day acute dietary LC<sub>50</sub> was >1076 mg Cu/kg diet (>4213 mg test material/kg diet). According to the US EPA classification, copper sulfate pentahydrate would be classified as practically nontoxic to canaries on an acute dietary basis. The active ingredient (Cu) would be classified as practically nontoxic up to 1278 mg Cu/kg diet.

There were 3 mortalities at the highest treatment level that are considered treatment related. There were decreases in body weight gain and feed consumption in the two highest treatment levels during the exposure period, but compensatory increased food consumption and weight gain were observed during the 3-day post exposure period. Consequently, birds in the two highest treatment groups exhibited “recovery” from the effects of the copper sulfate pentahydrate when they were fed clean food. This may or may not be applicable to exposure scenarios in the wild. Also, several birds exhibited a ruffled appearance during the exposure period, but all surviving birds appeared normal and healthy during the post-exposure period. The LC<sub>50</sub> of copper sulfate pentahydrate and copper were >4213 mg test material/kg diet and >1076 mg Cu/kg diet, respectively.

This study is **scientifically sound** and is classified as “**acceptable**”.

## **Results Synopsis**

Test Organism Size/Age(Mean Weight): 5-33 months; 16.7-26.5 g at test initiation

LC <sub>50</sub> : >1076 mg Cu/kg diet	95% C.I.: N/A
LC <sub>50</sub> : >4213 mg test material/kg diet	95% C.I.: N/A
Probit Slope: N/A	95% C.I.: N/A
NOAEC = 418 mg Cu/kg diet	

NOAEC based on decreases in body weight gain and feed consumption at top two treatment levels. Mortalities were observed at highest treatment level.

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## I. MATERIALS AND METHODS

**GUIDELINE FOLLOWED:** This study was conducted following guidelines outlined in the U.S. Environmental Protection Agency Series 850- Ecological Effects Test Guidelines OCSPP 850.2200. The following deviations were noted:

1. Guidance recommends young birds (5-10 days for mallard, 10-14 days for bobwhite). Birds in the definitive test were between approximately 5 and 33 months old at test initiation.
2. Only 10 birds were used in the control, though OCSPP guidance recommends at least 20.
3. Fasting was reported, though the duration was not specified.

These deviations do not affect the acceptability of the study.

**COMPLIANCE:** Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided. This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, 40 CFR Parts 160 and 792, 17 August 1989; OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF, 11 NohSan, Notification No. 6283, Agricultural Production Bureau, 1 October 1999, with the following exceptions: the characterization and stability of the reference substance under conditions of storage at the test site were not determined in compliance with GLP standards, and; periodic analyses of feed and water for potential contaminants were not conducted according to GLP standards, but were performed using a certified laboratory and standard U.S. EPA analytical methods.

### A. MATERIALS:

**1. Test Material** Copper Sulfate Pentahydrate

**Description:** Solid

**Lot No./Batch No. :** 8112013 (Lot No.)

**Purity:** 25.55% as Cu

**Stability of Compound Under Test Conditions:** Stable. Mean measured doses yielded recoveries ranging from 75 to 104% of nominal. Homogeneity samples collected from the 500 and 5000 mg test material/kg diet treatment groups yielded % recoveries of 67.4 and 88.7%, respectively, with CVs of 20 and 17%. Ambient stability of the test material after 5 days yielded recoveries of 87.8 to 124% of Day 0 doses.

**Storage Conditions of Test Chemicals:** Stored under ambient conditions

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## Physicochemical properties of Copper Sulfate Pentahydrate.

Parameter	Values	Comments
Water solubility at 20°C	Not Reported	
Vapor pressure	Not Reported	
UV absorption	Not Reported	
pKa	Not Reported	
Kow	Not Reported	

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

## 2. Test organism:

**Species (common and scientific names):** Canary (*Serinus canaria*)

**Age at study initiation:** ~5-33 months at test initiation

**Weight at study initiation (mean and range):** 16.7-26.5 g (based on all birds at test initiation)

**Source:** Maryland Exotic Birds, Pasadena, Maryland

## B. STUDY DESIGN:

### 1. Experimental Conditions

a. Range-finding Study: Nominal doses were selected based on a dietary probe study during which no mortalities occurred after 5 days at doses of 158 and 1580 mg test material/kg diet.

b. Definitive Study:

**Table 1: Experimental Parameters**

Parameter	Details	Remarks
		Criteria
<u>Acclimation</u>  Period: Conditions: (same as test or not) Feeding:   Health: (any mortality observed)	~6 weeks Not specified Commercially available canary food (Kaytee Forti-diet Pro Health canary and golden sunburst millet sprigs). During acclimation birds were transitioned to ZuPreem FruitBlend Flavor diet. Kaytee Hi Cal Grit was provided to aid with digestion. All birds appeared to be in good health at test initiation.	

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Parameter	Details	Remarks
		Criteria
Pen size and construction materials	Pens (Prevue Pet Products, Inc., Model No. F060) had floor space measuring 29 x 26 cm with a ceiling height of 31 cm. Walls, ceilings, and floors were constructed of coated wire. Pens were separated by a fiberglass barrier, and each pen contained perches and one cuttle bone.	<i>Recommended pen size is about 35 x 100 x 24 cm</i>
Test duration	8 days; 5 days of treated diet followed by 3 days of clean diet	<i>Recommended test duration is 5 days with treated feed and at least 3 days observation with "clean" feed.</i>
<u>Test concentrations</u> nominal:	500, 890, 1580, 2810, and 5000 mg test material/kg diet	The reviewer-corrected nominal doses were 128, 227, 404, 718, and 1278 mg Cu/kg diet. Mean-measured doses were 96, 234, 418, 635, and 1076 mg Cu/kg diet.
measured:	377, 916, 1635, 2485, and 4213 mg test material/kg diet	<i>Five or six test concentrations should be used in a geometric scale, unless the LC<sub>50</sub> &gt; 5000 mg ai/kg diet.</i>
<u>Solvent/vehicle, if used</u> type:	Corn oil 2%	
amount:		<i>Recommended solvents include distilled water, corn oil, propylene glycol, 1% carboxymethylcellulose, or gum arabic. The solvent should not be more than 2%.</i>
Diet preparation and feeding	Test material was mixed directly into the feed with 2% corn oil, blended, and mixed. A sufficient amount of diet was prepared at test initiation.	<i>The control group should be tested with a diet containing the maximum amount of vehicle used in treated diets.</i>
Feed withholding period	Fasting was reported, though the duration was not specified.	
Stability and homogeneity of test material in the diet determined (Yes/No)	Yes	
<u>Number of birds per replicate/groups</u> for negative control:	10	5 males and 5 females were included in each group
for vehicle control:	N/A	<i>The recommended number of birds per replicate is a minimum of ten.</i>
for treated:	10	
<u>Number of replicates/group (if used)</u>	Each bird was housed individually	

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Parameter	Details	Remarks
		Criteria
for negative control: for vehicle control: for treated:		
<u>Test conditions</u> temperature: relative humidity(%): photoperiod:	23.0-22.3°C 52-72% 16L:8D with 15-min transition periods of low-light intensity	<i>Recommended brooder temperature is about 35EC (95EF)</i> <i>Recommended room temperature is 22-27EC (71-81EF)</i> <i>Recommended relative humidity is 30-80%</i> <i>Recommended photoperiod is a minimum of 14 hours of light.</i>
Reference chemical, if used	N/A; a reference chemical was not used	

## 2. Observations:

**Table 2: Observations**

Parameters	Details	Remarks
Parameters measured (mortality/body weight/ mean feed consumption/ others)	-Mortality -Body weight -Feed consumption -Sub-lethal effects	
Indicate the stability and homogeneity of test chemical in the diet	Over the 5 day exposure period, mean-measured concentrations yielded overall recoveries of 75 to 104% of nominal. Homogeneity samples from the 500 and 5000 mg test material/kg diet groups yielded doses of 337 and 4433 mg test material/kg diet, with CVs of 20 and 17%, respectively.	
Indicate if the test material was regurgitated	No regurgitation reported.	
Treatments on which necropsies were performed	Necropsies were performed on all mortalities. Further, necropsies were performed on three birds from the control and each of the treatment groups.	

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Parameters	Details	Remarks
Observation intervals	Observations for mortality and sub-lethal effects were made daily. Body weights were measured at test initiation and on Days 1, 5, and 8. Food consumption was determined for Days 0-5 and 5-8.	
Were raw data included?	Yes	

**II. RESULTS AND DISCUSSION:**

**A. MORTALITY:**

No mortalities were observed in the control or mean-measured 96-418 mg Cu/kg diet treatment groups (377-1635 mg test material/kg diet). A single mortality was detected on Day 1 of the 635 mg Cu/kg diet (2485 mg test material/kg diet) treatment level, though this bird was found dead in the water dish and was not considered to be treatment-related. No other mortalities occurred in this treatment group during the exposure or post-exposure periods. Three birds (30%) died in the highest treatment group after 5 days of exposure to the treated food; no additional mortalities occurred during the post-exposure period. The study authors reported an LC<sub>50</sub> value of >5000 mg test material/kg diet based on uncorrected nominal values. This corresponds to measured doses of >1076 mg Cu/kg diet and >4213 mg test material/kg diet.



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**Table 3: Effect of Copper Sulfate Pentahydrate on Mortality of *Serinus canaria***

Treatment (mg Cu/kg diet/ mg test material/kg diet) Measured		No. of Birds per Treatment	Cumulative Mortality				
			Day 1	Day 3	Day 4	Day 5	Day 8
Control		10	0	0	0	0	0
96/377		10	0	0	0	0	0
234/916		10	0	0	0	0	0
418/1635		10	0	0	0	0	0
635/2485		10	1	1	1	1	1
1076/4213		10	0	1	1	3	3
LC <sub>50</sub>		>5000 <sup>a</sup>					
Reference chemical	mortality	N/A					
	LC <sub>50</sub>						
	NOEC						

<sup>a</sup> LC<sub>50</sub> value reported by the study authors was based on the uncorrected nominal dose.

## B. SUB-LETHAL TOXICITY ENDPOINTS:

There were no notable changes in body weight or body weight change in the three lowest treatment groups at any observation interval. The study authors reported that, while not significant, there was an apparent treatment-related decrease in body weight from Day 0 to 1 in the nominal 2810 mg test material/kg diet treatment group. During the same interval, there was a significant impact on body weight change in the nominal 5000 mg test material/kg diet treatment level (Dunnett's one-tailed test,  $p < 0.01$ ). In the 5000 mg test material/kg diet treatment group, body weight change from Days 1 to 5 and mean body weight on Day 5 significantly differed relative to the negative control. (Dunnett's one-tailed test,  $p < 0.05$ ). During the post-exposure period, there were significant compensatory weight gains in the top two treatment groups (Dunnett's one-tailed test,  $p < 0.01$ ).

Feed consumption was reduced in the top two treatment groups relative to the negative control during the exposure period. During the post-exposure period, feed consumption was greater in these two groups relative to the control suggesting a recovery from the treated feed during the exposure period.

All birds in the control and nominal 500 mg test material/kg diet treatment groups appeared normal and healthy throughout the test. There were a few instances of birds exhibiting a ruffled appearance, though nearly all surviving birds in the 5000 mg test material/kg diet treatment group had a ruffled appearance at some point during the exposure period. Across all treatment groups, all surviving birds appeared normal by Day 6.

The single mortality from the 2810 mg test material/kg diet treatment group was noted with a slightly pale liver. All three of the mortalities from the highest treatment group were thin, with a loss of muscle mass, a prominent keel, pale spleen and kidneys, and a primarily empty gastro-intestinal tract. Two of the three birds were noted with a small spleen and a pale liver. One of the three birds necropsied from the 890 mg test material/kg diet

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treatment level had a slightly pale heart, a distended proventriculus, a flaccid gizzard, and pale kidneys. None of the other birds randomly subjected to necropsy exhibited any remarkable findings.

**Table 4: Sublethal Effect of Copper Sulfate Pentahydrate on *Serinus canaria***

Treatment (mg Cu/kg diet/ mg test material/kg diet) Measured		Observation					
		Body Weight (g) (mean ± SD)				Food Consumption (g/bird/day) (mean ± SD)	
		Day 0	Day 5	Day 8	Total Change	Days 0-4	Days 5-7
Control		21.4±2.4	21.2±2.1	21.1±2.0	-0.4±0.6	3.7±0.3	3.0±0.3
96/377		20.3±1.1	20.3±1.4	20.6±1.4	0.2±0.4	3.5±0.3	3.0±0.2
234/916		20.2±2.2	19.9±2.2	20.4±1.9	0.1±0.7	3.4±0.4	2.9±0.4
418/1635		21.1±1.8	20.6±1.7	21.0±1.9	-0.1±0.9	3.5±0.3	3.1±0.4
635/2485		21.3±2.3	21.0±2.1	22.3±2.2	0.7±0.6	3.2±0.9	3.7±0.3
1076/4213		20.4±1.2	18.8±1.2	20.7±1.3	-0.1±0.7	2.7±1.1	3.5±0.3
EC <sub>50</sub>		N/A				N/A	
Reference chemical	NOAEC	N/A				N/A	
	EC <sub>50</sub>	N/A				N/A	

## C. REPORTED STATISTICS:

The LC<sub>50</sub> value was visually determined to be greater than the highest concentration. Body weight data were analyzed using Dunnett's Multiple Comparison test via TOXSTAT®. Toxicity values were based on the nominal, uncorrected concentrations (mg test material/kg diet).

## D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer visually estimated the LC<sub>50</sub> value to be greater than the highest measured concentration. Body weight change data and the associated standard errors were entered into CETIS statistical software version 1.8.7.12 with database backend settings implemented by EFED on 3/25/14. However, these data were not analyzed. The reviewer reported toxicity values based on the measured concentrations in terms of the test material (mg copper sulfate pentahydrate/kg diet) as well as copper only, (mg Cu/kg diet).

LC<sub>50</sub>: >1076 mg Cu/kg diet                      95% C.I.: N/A  
 LC<sub>50</sub>: >4213 mg test material/kg diet        95% C.I.: N/A  
 Probit Slope: N/A                                95% C.I.: N/A

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## E. STUDY DEFICIENCIES:

None.

## F. REVIEWER'S COMMENTS:

The reviewer's results were comparable to those of the study authors, with the exception that the reviewer used the measured concentrations corrected for the purity of Cu, whereas the study authors used the uncorrected, nominal concentrations. Therefore, the reviewer's results are reported in the Executive Summary and Conclusions sections of this DER.

To calculate the measured concentrations, the reviewer multiplied the analytical recoveries (%) of the test material (copper sulfate pentahydrate) with the nominal concentrations corrected for the purity of Cu (25.55%). Since the proportion of Cu is consistent in the test material, a recovery of 109% of nominal in 890 mg test material/kg diet treatment group would equate to a 109% recovery of the expected Cu concentration. The nominal 890 mg test material/kg diet level, corrected for the purity of Cu, would equal 227 mg Cu/kg diet, and a recovery of 109% on Day 0 would equal measured concentrations of 970 mg test material/kg diet and 248 mg Cu/kg diet. These calculations were performed for all treatment levels using recoveries from Days 0 and 5, and these values were averaged for the mean-measured concentrations.

The in-life portion of the definitive toxicity test was conducted from September 11 to 19, 2014.

## G. CONCLUSIONS:

The study is scientifically sound and is classified as "Acceptable". There were decreases in body weight gain and feed consumption in the two highest treatment levels during the exposure period, but compensatory increased food consumption and weight gain were observed during the 3-day post exposure period. Consequently, birds in the two highest treatment groups were "recovered" to control levels by the end of the study. Also, several birds exhibited a ruffled appearance during the exposure period, but all surviving birds appeared normal and healthy during the post-exposure period. The LC<sub>50</sub> of copper sulfate pentahydrate and copper were >4213 mg test material/kg diet and >1076 mg Cu/kg diet, respectively.

LC <sub>50</sub> : >1076 mg Cu/kg diet	95% C.I.: N/A
LC <sub>50</sub> : >4213 mg test material/kg diet	95% C.I.: N/A
NOAEC = 418 mg Cu/kg diet	
Probit Slope: N/A	95% C.I.: N/A

NOAEC based on decreases in body weight gain and food consumption during the exposure period of the study. Treatment related mortalities occurred at the highest treatment level.

## III. REFERENCES:

- Stephan CE. 1978. U.S. EPA, Environmental Research Laboratory, Duluth, MN. Personal Communication.
- Stephen CE. 1977. Methods for Calculating an LC<sub>50</sub>- Pages 65-84 in Aquatic Toxicology and Hazard Evaluations, American Society for Testing and Materials. Pub. No. STP 634. Philadelphia, PA.
- West, Inc. and DD Gulley. 1996. TOXSTAT® Release 3.5 Western Ecosystems Technology, Inc., Cheyenne, WY.